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Intact Coding Region of the Serotonin Transporter Gene in Obsessive-Compulsive Disorder

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Epidemiologic studies indicate that obsessive-compulsive disorder is genetically transmitted in some families, although no genetic abnormalities have been identified in individuals with this disorder. The selective response of obsessive-compulsive disorder to treatment with agents which block serotonin reuptake suggests the gene coding for the serotonin transporter as a candidate gene. The primary structure of the serotonin-transporter coding region was sequenced in 22 patients with obsessive-compulsive disorder, using direct PCR sequencing of cDNA synthesized from platelet serotonin-transporter mRNA. No variations in amino-acid sequence were found among the obsessive-compulsive disorder patients or healthy controls. These results do not support a role for alteration in the primary structure of the coding region of the serotonin-transporter gene in the pathogenesis of obsessive-compulsive disorder.

KEY WORDS: **serotonin transporter, platelets obsessive-compulsive disorder**

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a psychiatric illness characterized by repetitive, stereotyped intrusive thoughts and fears which can be temporarily relieved by performance of compulsive behaviors. Epidemiologic studies indicate that OCD is heritable in some families [Pauls et al., 1995]. To date, however, no genetic abnormalities have been identified in individuals with OCD.

Because individuals with OCD selectively respond to chronic treatment with serotonin reuptake-inhibiting antidepressants and not other antidepressants [Greist et al., 1995; Murphy et al., 1995], the serotonin transporter is a candidate gene for molecular genetic investigations in this illness. The serotonin transporter plays a critical role in the termination of serotonergic neurotransmission by sodium-dependent uptake of serotonin into the presynaptic neuron [Kanner and Schuldiner, 1987; Amara and Kuhar, 1993; Barker and Blakely, 1995], and changes in serotonin-transporter gene expression have been noted after chronic treatment with serotonin-reuptake inhibitors [Lesch et al., 1993a]. Thus, a genetic alteration in the serotonin transporter could play a predisposing or pathophysiological role in OCD. There is variability among OCD patients in the therapeutic response to serotonin-reuptake inhibitors, which would be consistent with the apparent genetic heterogeneity of the illness [Pauls et al., 1995]. Studies of platelet serotonin uptake and ^{3}H-imipramine and ^{3}H-paroxetine binding to platelets in OCD patients have produced mixed results [Bastani et al., 1991; Vitiello et al., 1991], which would also be consistent with genetic heterogeneity. Since twin studies indicate that platelet serotonin uptake and platelet imipramine binding are under genetic control [Friedl and Propping, 1984; Meltzer and Arora, 1988].

The human serotonin transporter cDNA has recently been sequenced [Lesch et al., 1993b; Ramamoorthy, Bauman et al., 1993) and the gene localized to chromosome 17 [Ramamoorthy et al., 1993; Gelernter et al., 1995]. The primary structure has been found to be identical in samples derived from human platelets and from the human midbrain raphe complex [Lesch et al., 1993b]. The serotonin transporter sequence also has been reported to be identical in human brain and in a human placental trophoblastic cell line [Ramamoorthy et al., 1993]. Sequence analysis predicts a 630 amino acid protein with 12 transmembrane segments. Based on similarities and differences among monoamine transporters and other cell-membrane transporters, several transmembrane regions (1, 7, 11, and 12) have been identified as likely sites for recognition of serotonin and other ligands [Kitayama et al., 1992; Amara and Kuhar, 1993; Barker et al., 1994].

In this study, we used direct sequencing of platelet-derived cDNA to look for changes in the coding region of the serotonin-transporter gene in OCD patients as compared to control subjects.

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MATERIALS AND METHODS

Subjects

Twenty-two patients who met DSM-III-R [American Psychiatric Association, 1987] criteria for OCD (11 women and 11 men; mean ± SD age, 37 ± 11 years) participated in the study. Patients were enrolled in ongoing research protocols at the OCD outpatient research clinic at the National Institute of Mental Health. Patients were diagnosed by consensus, using the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer et al., 1990] administered by a trained clinician, a clinical interview administered by a research psychiatrist, and Yale-Brown Obsessive-Compulsive Disorder Scale (YBOCS) [Goodman et al., 1989] and the Hamilton Depression and Hamilton Anxiety Ratings [Hamilton, 1967] administered by a second psychiatrist. Mean duration of illness was 15 ± 12 years. Ten of the 22 patients had a positive family history of OCD, and none reported a family history of Tourette syndrome or tic disorder. Four patients had current or past history of tic disorder, and none had a history of Tourette syndrome. All OCD patients were Caucasian.

Control samples (N = 4) were from Caucasian medical student volunteers who were medically healthy and had no personal history of psychiatric illness, as determined by a structured psychiatric interview. These subjects also had no history of psychiatric illness in any first-degree relatives. All patients and controls were unrelated individuals.

Preparation of Platelet RNA

Blood platelets from patients and controls were isolated by differential centrifugation. Ten-ml blood samples were collected in iced tubes containing EDTA and were centrifuged immediately at 150*g* for 20 min at 4°C. The platelet-rich plasma was resuspended in Ringer's citrate dextrose buffer without $MgCl_2$ and recentrifuged at 550*g* for 30 min at 4°C. The platelet pellet was frozen at −80°C prior to RNA isolation. Total RNA was isolated using guanidine thiocyanate-phenol-chloroform [Chomczynski and Sacchi, 1987]. Platelets were homogenized in 4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.5, 0.5% sarcosyl, and 0.1 M β-mercaptoethanol. After acidified phenol/chloroform extraction and isopropanol precipitation, the RNA was washed in 75% ethanol, quantitated at 260 nm, and stored at −80°C.

Sequence Analysis

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of a large cDNA fragment (approximately 2,000 base pairs), representing the entire coding region of the serotonin transporter and three smaller overlapping cDNA fragments (approximately 700–800 base pairs), together also representing the entire coding region of the serotonin transporter, was performed as previously described [Lesch et al., 1995]. The three smaller cDNAs were used for subsequent sequence analysis using the dideoxynucleotide chain termination method. Nucleotide sequences were aligned and analyzed using MacVector software (IBI).

RESULTS

Direct PCR-sequencing of the serotonin-transporter protein-coding region in the cDNA sequence synthesized from platelet serotonin-transporter mRNA failed to reveal changes in the primary structure of the serotonin transporter in this sample of patients with OCD as compared to controls.

DISCUSSION

The analysis of the primary structure of the serotonin transporter in 22 patients with OCD by PCR amplification and direct sequencing of cDNA synthesized from platelet mRNA did not show differences from healthy controls. This finding indicates that the selective response of patients with OCD to treatment with pharmacologic agents that block serotonin reuptake through the serotonin transporter is not a consequence of OCD-related abnormalities in the DNA coding for the serotonin transporter.

A limitation of the PCR-based amplification and direct sequencing methods used in this study is that changes in regions of the gene which are not transcribed, such as splice junctions and promoter or enhancer regions, cannot be detected [Sommer, 1992]. These regions, which were not transcribed and thus not examined in this study, may play an important role in the regulation of serotonin-transporter production and function. Although our data indicate no change in the primary structure of the serotonin transporter in OCD patients, it remains to be determined whether abnormalities in regulation of expression of the serotonin-transporter gene contribute to the pathophysiology or the selective treatment response of the illness. In addition, the human serotonin transporter has several structural motifs that predict extensive posttranslational modification [Lesch et al., 1993b,c], and it is possible that posttranslational modifications are altered in patients with OCD.

It is also possible that changes in the coding region of the serotonin transporter are associated with OCD in some individuals or some families which were not included in our sample. OCD as defined by DSM-III-R is likely to have several modes of pathogenesis. For example, OCD is known to result from brain lesions in some individuals, and to have a more strongly familial component in others [Pauls et al., 1995]. Relatively rare polymorphisms could easily be missed in a sample of this size. One prior study of the serotonin transporter in 17 depressed patients which also used direct sequencing of platelet mRNA-derived cDNA revealed one silent single-base substitution in one patient [Lesch et al., 1995].

Other candidate gene studies of OCD patients have also failed to find any differences from control subjects. One prior direct-sequencing study did not detect any difference in the molecular structure or distribution of the dopamine D3 receptor *Msc*I polymorphism in a large number of patients with OCD as compared to controls [Catalano et al., 1994]. Another study in OCD patients using denaturing gradient gel electrophoresis (DGGE) found no structural changes compared to con-

trols in three dopamine D2 receptor exons encoding for the third intracytoplasmic section of the D2 receptor, believed to be critical for G protein coupling [Novelli et al., 1994]. That study also did not find a difference between OCD patients and controls in the frequency of a previously described polymorphism in exon 6 [Novelli et al., 1994]. In addition, a linkage study carried out in a large kindred multiply affected with Tourette syndrome, chronic motor tics, and OCD found no association between Tourette syndrome and variations in the serotonin 5HT1A receptor or tryptophan oxygenase genes [Brett et al., 1995].

In conclusion, although we cannot exclude a defect in serotonin-transporter activity in an as-yet unexamined kindred of OCD patients or changes in activity of regulatory genes controlling expression of the serotonin-transporter gene, our finding of a normal sequence in the coding region of the serotonin-transporter gene in all 22 patients in this study provides preliminary evidence that alterations in the primary structure of the serotonin transporter are not generally involved in the pathogenesis of OCD.

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